


25. (Twice Amended) A method of producing germline chimeric avians

comprising:

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- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
 - (ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);
 - (iii) transferring said PGCs into a recipient avian embryo; and
 - (iv) obtaining germline chimeric avians having germline cells that have the genotype of said PGCs.

26. (Thrice Amended) A method of producing germline and somatic cell chimeric avians which comprises:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF),for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring said EGs into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;

- (iv) allowing said recipient avian embryo to develop into a germline and somatic cell chimeric avian having germline and somatic cells that have the genotype of said PGCs.

27. (Twice Amended) A method for producing avian embryonic germ (EG) cells comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
(ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

- (1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and
(4) insulin-like growth factor (IGF)

so that avian EG cells are produced.

28. (Twice Amended) A method for producing a chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
(ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:

- (1) leukemia inhibitory factor (LIF),
(2) basic fibroblast growth factor (bFGF),
(3) stem cell factor (SCF) and
(4) insulin-like growth factor (IGF);

- (iii) transferring said PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;

- (iv) allowing said recipient avian embryo to develop into a chimeric avian.

29. (Twice Amended) A method for producing a germline chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);
- (iii) transferring said PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs; and
- (iv) allowing said recipient avian embryo to develop into a germline chimeric avian.

30. (Twice Amended) A method for producing germline or somatic cell chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);